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PRINCIPAL INVESTIGATORS: Lawrence E. Armstrong, Ph.D.

CONTRACTING ORGANIZATION: University of Connecticut
Storrs, Connecticut 06269-1110

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6. AUTHOR(S)
Lawrence E. Armstrong, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Connecticut
Storrs, Connecticut 06269-1110
e-mail:
armstron@uconnvm.uconn.edu

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13. ABSTRACT ***(Maximum 200 Words)***

The purpose of this investigation was to discover the effects of a physical training plus heat acclimation program on exercise performance, thermoregulation, immune function, and reproductive and stress hormone responses in three groups of women: oral contraceptive (ORAL), Depo-Provera (DEPO) contraceptive, and eumenorrheic-ovulatory (EU-OV) no contraceptive. The three-year total number of subjects was 36 (EU-OV, 14; ORAL, 15; DEPO, 7). All subjects were stronger, more physically fit, leaner, and heat acclimated at the end of this 8-week study. The multiple stressors provided by eight weeks of physical training and heat exposure had no important effects on either menstrual or hormonal status. Plasma epinephrine concentrations were unchanged among groups or across time. Plasma norepinephrine concentrations also were unchanged among groups, however the ORAL group showed a significant increase (pre-exercise to post-exercise) during the pre-acclimation testing, and pre-exercise values were significantly lower as a result of physical training. Resting aldosterone concentrations were unremarkable, with similar concentrations among groups and across time. Immunological analyses suggested that the effect of oral contraceptives on the immune system is small or physiologically unimportant. In summary, the effects of oral and injectable contraceptives on all blood and physiological variables were minimal.

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Lawrence F. Armstrong 10/21/99
PI - Signature Date

Carl M. Marsh 10/21/99
PI - Signature Date

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Introduction

It is important that an encounter with simultaneous **multiple stressors** has been recognized as a prominent etiologic factor in military casualties (23,53). The goal of this study was to provide information to reduce complications associated with stressful environments and therefore decrease casualties in military women. Comprehensive information about the health of military women facing multiple stressors is not currently available. This study was designed to clarify the ability of exercise training and heat acclimation to minimize the effects of multiple stressors on (a) exercise responses in the heat while dehydrated, (b) immunocompetence, and (c) hormone levels. Three groups of young women participated in all health and hormone screening procedures, eight weeks of physical training/heat acclimation, and exercise-heat tolerance testing. Members of each of these three groups (oral contraceptive users, n = 15; injectable contraceptive users of Depo Provera, n = 7; and control subjects who were eumenorrheic and ovulatory, n = 14) were tested during this 3-year study.

Background

Of the 340,000 women in the Armed Forces of the United States, two-thirds (200,000) serve on active duty and are between the ages of 18 and 30 years. Although they are healthy and active, the unique challenges that military women face (i.e., basic training, physical training, combat) far exceed those in civilian occupations. For example, deployment introduces many unique physical stressors including harsh environments, primitive housing/sanitary standards, and exposure to novel diseases; as well as psychological stressors (e.g., close quarters) that may affect heat tolerance and immune function.

It is important that an encounter with simultaneous multiple stressors has been recognized as a prominent etiologic factor in military casualties (23,53). For example, concurrent multiple stressors such as a sudden increase in physical training, fever or disease, dehydration, and a lengthy heat exposure were reported during the 5 days prior to exertion heatstroke in 10 soldiers (5).

CONTRACEPTIVE USE BY MILITARY WOMEN

Because military women share quarters with men, stressors related to sexuality arise. These include lack of privacy, a four-to-one ratio of males to females, sexual harassment, and unplanned pregnancy (18). At any point in time, approximately 18,000 active-duty military women are pregnant (61). The majority of these pregnancies are unplanned, in women under the age of 25 (67 %) (25). The need for contraception in the U.S. Armed Forces is authentic, considering individual career advancement, financial resources and mission priorities.

It is difficult to determine accurate statistics regarding the number of military women who use contraceptives (personal communication, LTC Katy Reynolds M.D., Nov 1995). But, it is known that 56% of military women who experience unplanned pregnancy use some form of contraceptive (25). Medical publications (57,59) indicate that 95 % of all sexually active civilian women aged 15 - 44 years, and 74 % of sexually active college females, use some form of contraception in the United States.

A woman's choice of contraceptive method is affected not only by the perceived efficacy and convenience of the technique, but whether additional risks or benefits are associated with its use. While oral estrogen and progestin contraceptive therapies remain the most popular method of pregnancy prevention in the United States, little is known about their effects on exercise performance, thermoregulation, and immune function.

The use of long-acting contraceptive methods is increasing in the U.S. Armed Forces because they simplify compliance. For example, Depo-Provera (depot medroxyprogesterone acetate), a long-acting (3-6 months) injectable agent, has an extremely low failure rate (0.0-1.2 per 100 woman-years) and is used by 11 million women in over 90 countries, including the United States (22,58). The U.S. Food and Drug Administration approved its use in 1992, based on WHO epidemiologic data.

Depo-Provera typically provides a three month window of safe and effective contraception, and is ideal for use in military settings (i.e., basic training, deployment, combat). One injection provides a female soldier with three months of uncomplicated birth control. This contraceptive technique is worthy of study because it is used by an ever-increasing percentage of military women and can be administered safely, with little or no supervision, for many years. Although Depo-Provera is the most widely studied injectable steroid formulation (over 500 investigations involving its effectiveness and safety have been published since it became available 29 years ago), very little is known about its effects on exercise performance, thermoregulation, or immune function.

ORAL CONTRACEPTIVES, IMMUNE FUNCTION, AND
RESPONSES TO EXERCISE-HEAT-DEHYDRATION

Thermal balance may be altered by phase of the menstrual cycle, probably due to increased progesterone levels during the luteal phase (40). For example, exercise during the **luteal** phase is characterized by a higher T_{core} (0.4°C) and a higher T_{re} sweat threshold temperature (0.25°C), versus the **follicular** phase (31,40). Although these minor effects have minimal military relevance, they become more important if ambient conditions are hot and humid, and if exercise is intense and prolonged. One study demonstrated, for example, that a 0.6°C T_{re} difference (luteal versus follicular phase) occurred when women exercised for 60 minutes at 60% VO_2max in a 22°C and 60 % rh environment (46). Had the ambient temperature been 35-40°C, the difference between luteal and follicular phase responses would probably have been greater. Admittedly, these minor effects are not as militarily relevant as the effects of oral contraceptives on thermal balance and exercise performance. Although little is understood, it has been shown that oral contraceptives users exhibit more uniform T_{core} and sweating responses than non-users, probably because there is no phasic alteration of **progesterone** levels in these women. Further, injected progesterone (i.e., Depo-Provera) increases basal T_{core} within 24-36 hours of drug administration (31). The impact of these findings on heat tolerance may have significant implications for military women in basic training, deployment, or combat settings and we are examining these issues.

Most military stressors lead eventually to a common response pathway involving activation of the sympathetic nervous system, the secretion of cortisol and epinephrine. The hypothalamic response begins with secretion of corticotropin-releasing hormone (CRH). CRH stimulates secretion of **immunostimulants** (prolactin, LH, FSH, TSH, growth hormone) and **immunosuppressors** (beta-endorphin, ACTH, cortisol, and alpha-melanocyte stimulating hormone). Plasma cortisol, which is elevated during stress, decreases the levels of antibodies and leukocytes, depresses the ability of white blood cells to digest phagocytized substances, and reduces fever. The degree to which these systems are activated depends on the total stress encountered, the previous physical and mental experiences of the individual, and the degree of control that she or he can exert over the stressful situation (32).

The mechanisms by which these contraceptives affect reproduction are described below (59).
Combined estrogen/progestin formulations: Both estrogen and progestin prevent ovulation by suppression of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion. This occurs via inhibition of hypothalamic gonadotropin-releasing hormone (GnRH) release. As a result of the hypothalamic-pituitary suppression of GnRH, LH and FSH, endogenous progesterone and estradiol also are suppressed. Besides the inhibition of ovulation, cervical mucus composition is altered and ovum transport/implantation are modified (59).
Depo-Provera: A steroid that prevents follicular maturation, ovulation, and endometrial thickening by inhibiting the secretion of gonadotropins. Depo-Provera is similar in structure to naturally occurring progesterone. Contraceptive plasma levels of this compound are reached within 24 hours of injection and are sustained for 14 weeks after injection (38). Plasma levels of estradiol remain within the normal range (22,38).

Changes in the plasma levels of compounds relevant to reproduction and fertility may alter immune function in women. Elevated **estrogen** levels result in general immunosuppression (8,60) and decreased natural killer cell activity (8). High concentrations of estradiol also serve to suppress immune function in women (41). In contrast, low circulating estrogen is associated with increased total lymphocytes and CD_4^+ counts (34). Therefore, **oral contraceptives** (which contain both estrogen and progesterone) should affect the immune system differently from **Depo-Provera** (which acts similarly to progesterone) because various immune cells are affected differentially by estradiol and progesterone (41). Depo-Provera, theoretically, should enhance immune function, in comparison to oral contraceptives. It is not known, however, if such alterations are sufficient to cause illness, or if physical training and heat acclimation will affect specific components of the immune system. Species differences exist; rats and mice exhibit responses that are opposite to those described above for humans (1,42).

HEALTH & PERFORMANCE OF MILITARY WOMEN: RESISTANCE TO INFECTION

A robust immune system (i.e. a high level of total IgG and a high titre of anti-LPS IgG, see below) is desirable for soldiers at all times. Conversely, the combination of overtraining and the stress of new surroundings suppresses immune function and often is blamed for illnesses (i.e. the common cold, sore throat, influenza, mononucleosis) that afflict soldiers and athletes during physical training (52).

The complexities and redundancies of the immune system (16), as well as the many differences in protocols of published studies (62), have contributed to a long-standing polarity of opinion regarding the influence of **acute exercise** and long-term **physical training** on immune function. Although it is known that both women and men show a marked leukocytosis (total white blood cells and polymorphonuclear neutrophils) following exercise of greater than 3 hours duration (63), few definitive conclusions are possible (16,55,62). Similarly, immunologic responses to other stressors inherent in military training (i.e., **dehydration, heat stress**) have not been investigated adequately in women. This is relevant to the present investigation because military training, especially for new inductees or field units that are deployed to stressful environments, subject soldiers to **multiple stressors** in virtually all cases (37). These scenarios and multiple stressors may affect temperature regulation and other vital bodily processes negatively in women (24,45).

SOLDIER HEALTH AND PERFORMANCE: INTESTINAL VIGOR

In addition to immune function, the preceding scenarios illustrate the military relevance of normal nutrient delivery (especially water, salt, and carbohydrates) during physical training (7) and combat (3). However, the research of Gaffin and colleagues (12,15,27,28) and the review of Hubbard et al. (36) have revealed an immunological response to intestinal events, during concurrent multiple stressors, that is not widely appreciated.

During digestion, gram negative bacteria exist in chyme in the small and large intestines. Dead gram negative bacteria provide large amounts of the toxic cell wall component **lipopolysaccharide (LPS)**. LPS found in the outer membrane of gram-negative bacteria are known as **endotoxin**. High levels of plasma LPS seem to be a proximate cause of human septic shock (43). One of the most important discoveries in critical care medicine in the 1980s involved the recognition that LPS may leak out of damaged intestines into the blood, resulting in cardiovascular insufficiency, extensive organ damage or death, in severe cases.

THE PATHOGENESIS OF ENDOTOXEMIA

When LPS enters the portal circulation, one of three fates is possible: (a) detoxification by Kupffer's cells in the liver, (b) inactivation by binding to circulating factors (i.e., HDL, anti-LPS IgG, LPS binding protein, CD-14 or soluble CD-14 receptor), (c) expression of toxicity by binding to LPS binding protein (LBP) and subsequently to CD-14 receptors on the membranes of microphages and other cell types (28). This latter fate results in hypersecretion of **cytokines** (e.g., TNF, IL-1), toxic immune mediators that may cause fever, nausea, vomiting, diarrhea, headache, tissue injury, shock, or death. These symptoms are observed in many cases of heatstroke (35), and have led authorities to suggest that cytokine release is a risk factor for exertional heatstroke (36). Although heatstroke is unheralded and has an unknown etiology in most cases, autopsies of human heatstroke victims have found high titres of plasma LPS and cytokines (13,14). LPS also could be involved in heatstroke by suppressing sweating (9) or cardiac function (44).

Cytokines may alter soldier performance in other ways (23): (a) Both TNF and IL-1 can induce slow wave sleep, suppress appetite (39,54), and cause fever by stimulating prostaglandin E_2 synthesis (21). (b) TNF can induce all features of endotoxin-induced septic shock (43). (c) IL-1 changes the response of arteries to norepinephrine in different vascular beds, and may cause abnormal regional blood flow (47).

EXERCISE-HEAT STRESS, SPLANCHNIC ISCHEMIA, AND LPS

Compared to exercise in cool environments, exercise-heat stress produces a markedly reduced blood flow in splanchnic vascular beds concurrent with an increased heart rate (48). This diversion of blood flow contributes to increased skin blood flow (important for heat dissipation), but carries the threat of compromising the function of splanchnic organs (49,50). This is important because the removal of bacteria and other microorganisms is normally a function of the reticuloendothelial system (RES) in the liver (2). The splanchnic ischemia that accompanies sustained hyperthermia during exercise also has been proposed as a cause of heat exhaustion (6) and the intestinal illnesses seen in 20-30% of all marathon runners (11). If exercise-heat stress or ischemia is great, an increase in plasma LPS may occur due to increased gut permeability. This phenomenon has been observed in

primates, cats, miniswine, and rats (28,51). These animals demonstrated that core **hyperthermia** must reach severe levels (42 - 45°C) before lethal increases in LPS occur (28,29,36), but low levels of LPS may enter the circulation at a rectal temperature as low as 39°C (29).

Other stressors enhance the entry of gut-derived LPS into the circulation: hypovolemia, splanchnic artery occlusion, and diarrhea (28,36). Hypoxia also has been shown to potentiate the production of TNF and IL-1 in human blood mononuclear cells, after resting exposure to subthreshold levels of LPS (30).

Human studies suggest that **exercise and/or physical training** play an important role in endotoxemia. For example, plasma LPS levels were elevated after strenuous exercise by triathletes and ultramarathon (89.5 km) competitors: (a) Bosenberg et al. (12) found that LPS rose and the "natural" anti-LPS IgG (the antibody formed in response to LPS) decreased during competition; (b) Brock-Utne et al. (15) observed that 80 % of collapsed runners had elevated levels of plasma LPS. The casualties with low/normal levels of LPS, but high levels of anti-LPS IgG, symptoms were far less severe than those with high/abnormal plasma LPS, and low levels of anti-LPS IgG; this latter group required two days to recover. Thus, the presence of a higher titre of anti-LPS appeared to protect the runners, possibly because they had been autoimmunized during daily training.

A critical question has emerged from these human studies. Can the level of "natural" anti-LPS antibodies be manipulated in soldiers to effectively reduce susceptibility to heat illness? The hypothetical answer suggests that part of the benefit of **physical training** for soldiers might be to increase the natural plasma levels of anti-LPS IgG. This could occur as small amounts of LPS enter the circulation, during strenuous training, on a daily basis. Because LPS stimulates a hypersecretion of the cytokines TNF and IL-1, this issue has great military relevance because cytokines may increase casualty rates (see above) and the susceptibility to heat illness (36).

Purpose / Technical Objectives

It was essential to the goals of this research project that we meticulously controlled the onset of testing and training for each subject's menstrual phase and status, and documented compliance to contraceptive therapies.

Because the effects of oral and injectable contraceptives on physical training and heat acclimation are virtually unknown, and because the immune system maintains a constant state of personal health by interacting with every organ system in the body, the following technical objectives and hypotheses have great relevance to military women and military units.

A. Primary Longitudinal Objectives

1. To evaluate differences among the three groups with respect to immune function before and after an eight-week training/heat acclimation program.
2. To evaluate differences among the three groups with respect to the exercise-heat tolerance test (EHT) responses before and after an eight-week training/heat acclimation program.
3. To evaluate differences among the three groups with respect to reproductive hormone status before and after an eight-week training/heat acclimation program.

B. Secondary Longitudinal Objectives

1. To evaluate differences among the three groups with respect to stress hormones before and after an eight-week training/heat acclimation program.
2. To evaluate differences among the three groups with respect to body composition and VO_2max before and after an eight-week training/heat acclimation program.

C. Dependent Variables: Categorical Definitions

1. Reproductive hormone - estradiol, progesterone, sex hormone binding globulin
2. Immune function - CD-4+, CD-8+, anti-LPS IgG, total IgG, HSP_{70}, IL-10, IFNg

(abbreviations defined on page 13)

3. Exercise-heat tolerance -
   a) thermoregulatory markers: rectal temperature, skin temperature, whole body & local sweat rate, skin blood flow
   b) fluid-electrolyte balance: aldosterone, osmolality, hematocrit, hemoglobin, plasma volume shift
   c) exercise performance: heart rate, blood pressure, exercise tolerance time, rating of perceived exertion, rectal temperature, glucose, lactate
4. Stress hormones - cortisol, epinephrine, norepinephrine

D. Independent Variables:

A. groups: oral contraceptive users (ORAL), Depo Provera users (DP), eumenorrheic ovulatory women taking no form of birth control (EU-OV)
B. time: pre-training/heat acclimation
post-training/heat acclimation

Null Hypotheses

A. Null Hypotheses Associated with Primary Longitudinal Objectives

1. There will be no significant differences among the three groups with respect to immune function before and after an eight-week training/heat acclimation program. We expect that the Depo-Provera group will exhibit the most favorable immune response.

2. There will be no significant differences among the three groups with respect to the exercise-heat tolerance test (EHT) responses before and after an eight-week training/heat acclimation program. We expect that the Oral Contraceptive group will exhibit the most favorable thermoregulatory response.

3. There will be no significant differences among the three groups with respect to reproductive hormone status responses before and after an eight-week training/heat acclimation program. We expect that the EU-OV group will exhibit the greatest perturbations in reproductive hormone status.

B. Null Hypotheses Associated with Secondary Longitudinal Objectives

1. There will be no significant differences among the three groups with respect to stress hormones before and after an eight-week training/heat acclimation program. We expect that the magnitude of the changes in stress hormone levels will be equivalent among the groups.

2. There will be no significant differences among the three groups with respect to body composition and maximal aerobic power (VO_2max) before and after an eight-week training/heat acclimation program. We expect that the changes in body composition and VO_2max will be equivalent among the groups.

Body of the Final Report

Statement of Work / Experimental Scope

Technical Objective: To evaluate the effects of oral and injectable contraceptives on hormones (i.e., reproductive, fluid-electrolyte, stress), immune system function, and exercise-heat-dehydration tolerance.

Task 1: Months 1 - 4: Order supplies, materials; prepare equipment and chamber. Oversee graduate students/ technicians and budgetary matters. Insure that research meets regulations of Environmental Health & Safety and the Institutional Review Board for Human Subjects.

Task 2: Months 4 - 7: Recruit, screen, identify, and brief test subjects. Conduct preliminary screening to eliminate subjects with exclusionary criteria.

Task 3: Month 8: Conduct Intensive screening of subjects. Select >15 subjects in three groups: oral contraceptive users, Depo-Provera users, and EU-OV. Collect descriptive subject data.

Task 4: Month 8: Collect two baseline blood measurements of immune system markers and hormones (reproductive, fluid-electrolyte), verify normalcy of reproductive function, and verify menstrual phase timing.

Task 5: Month 8: Prepare environmental chamber and instruments for testing. Conduct >15 exercise-heat tolerance tests (90 min each) at 38°C, to document pre-training responses.

Task 6: Months 8 - 10: Conduct eight-week training program for >15 women (6 days/week; 3 days involve heat exposure up to 90 min).

Task 7: Months 8 - 10: Collect blood samples throughout training period, to evaluate hormones (reproductive, fluid-electrolyte), immune system function, normalcy of reproductive function, and menstrual phase timing.

Task 8: Month 10: Prepare environmental chamber and instruments for testing. Conduct >15 exercise-heat tolerance tests (90 min each) at 38°C, to evaluate post-training responses. Collect blood samples to identify post-training levels of hormones, immune markers, and timing of menstrual phases.

Task 9: Months 10 - 12: Laboratory analyses. Enter data into spread sheet. Submit annual report when required.

Tasks 10 - 17: Months 13 - 24: Repeat Tasks 1 - 8 above, to begin collecting data on >15 additional women, bringing the number to 30 total subjects.

Tasks 16 - 25: Months 25 - 36: Repeat Tasks 1 - 8 above, to begin collecting data on >15 additional women (goal: bring the number to 15 in each treatment group).

Task 26: Submit final report when required.

Task 27: Prepare abstracts for submission to scientific conferences. Prepare manuscripts for submission to journals for publication.

Experimental Methods and Procedures

This section presents the experimental design and procedures which were used to meet study objectives. Three groups of young women participated in all health and hormone screening procedures, eight weeks of physical training/heat acclimation, and exercise-heat tolerance testing. Members of each of these three groups (oral contraceptive users, n = 15; injectable contraceptive users of Depo Provera, n = 7; and control subjects who were eumenorrheic and ovulatory, n = 14) were tested during each year of this 3-year study.

SUBJECT CHARACTERISTICS

Female civilian students attending the University of Connecticut were recruited by announcements posted on bulletin boards, in classrooms, and the daily campus newspaper. The University of Connecticut has approximately 8,000 female students within the 18-34 year age range, and we are experienced in recruiting women for experimental and training studies. University staff and women living in adjacent communities also were recruited. Potential volunteers were given a description of the objectives, procedures, risks, and time commitments required for the study. All subjects were asked to provide written voluntary consent to participate, in compliance

with the Institutional Review Board for Human Subjects at The University of Connecticut. Interested subjects completed a medical history and physical activity questionnaires, and were interviewed by one of the investigators.

All subjects were required to meet the following criteria:

a) aged 18 to 34 years;
b) within the average range (± 2 SD) of U.S. military women for height (162 ± 13 cm) and weight (60 ± 16 kg);
c) in good health, as determined by a medical and gynecological examination (private physician, within the previous 12 months) including a normal Papanicolaou smear;
d) free of any chronic disease including thyroid disease and hyperprolactinemia;
e) lack of any recent (within three months) changes in menstrual status;
f) appropriate activity history;
g) no history of eating disorder or depressive illness within the past three years and an appropriate score on the Eating Disorders Inventory (EDI);
h) the absence of any contraindications revealed in a medical history that might preclude participation in the study, including a history of heat-related illness, endotoxemia, chronic respiratory disorder, cardiovascular disease, hypertension, metabolic disorders, convulsive disorders, drug or alcohol dependence; and
i) not routinely taking a prescription or over-the-counter medication that would alter variables measured herein.

All subjects were non-pregnant, during all screening and testing,, as determined by blood sample analysis for HCGH. All University of Connecticut students were required to have current inoculations before matriculating.

Subjects were asked to report any gastrointestinal or respiratory tract illnesses, or superficial injuries (i.e. abrasions, cuts) incurred during their involvement in the project. All subjects were performing no more than 90 minutes of aerobic activity per week for the previous 12 months, and had a maximal oxygen uptake ($V0_2$ max) less than 42 $ml \cdot min^{-1} \cdot kg^{-1}$. Subjects were paid for their participation.

EXPERIMENTAL DESIGN

To test the various hypotheses set forth in this investigation, we utilized three groups of women: a) females currently ingesting an oral contraceptive (ORAL) for a minimum of three months prior to the study (n = 15), b) females receiving depot medroxyprogesterone acetate (DP; Depo-Provera) long-acting contraceptive therapy for a minimum of three months prior to the study (n = 15), and c) eumenorrheic ovulatory (EU-OV) females (n = 15).

During the Fall semester of academic years 1996-97, 1997-98 and 1998-99 participants were recruited and underwent a two month (during the months of October, November and December) preliminary screening procedure. Beginning in January, subjects performed an intensive one-month screening procedure, followed by an 8-week exercise training plus heat acclimation program. Exercise training sessions were held six days per week, with the heat acclimation sessions (alternate days, up to 90 $min \cdot day^{-1}$) comprising three of those days. Maximal oxygen uptake tests and exercise-heat tolerance (EHT) tests were performed prior to, and at the end of the 8-week training program. It is our contention that a non-training control group was not necessary for this study for two reasons: a) because it represents a scenario with little or no military relevance and b) the pre- and post-training measurements for all groups in this study allow us to compare untrained and trained states.

Weight, menstrual patterns, training habits, and any atypical stressors were monitored throughout the study. Subjects were weighed (kg) during all laboratory visits. Menstrual bleeding patterns and exercise reports were monitored daily via diary and training cards. Subjects recorded any medications that they were consuming in their menstrual diary. Seven day nutritional dietary records were completed during the first seven days of each menstrual cycle (or 28 day period) to make sure that dietary intakes were appropriate to support the nutritional demands and caloric expenditure of training. Thus, any significant changes in dietary habit were documented as thoroughly as possible. Each subject's health status was of great concern to us, from an ethical perspective, and to ensure that she continued with the training program. The Women's Health Center on our campus was available for appointments and to collect clinical data concerning illness.

MENSTRUAL CYCLE AND COMPLIANCE MONITORING

All eumenorrheic ovulatory (EU-OV) subjects were asked to participate in menstrual screening procedures that accurately determined their ovulatory status and the length and adequacy of their luteal phase. These were determined via blood samples that pinpointed the onset of the luteinizing hormone (LH) surge to within 12-24 hours. Subjects were asked to maintain menstrual logs to document menstrual cycle length and duration of menstrual flow days prospectively. During the menstrual cycle immediately preceding training, and during the

second month of physical training, all eumenorrheic subjects had blood sampled during the early follicular phase (days 2 - 6). Approximately 7 days thereafter, daily blood draws were completed until 1 day after the peak LH concentration had been reached (e.g., ovulation). Finally, approximately 7 days after the peak LH level, an additional blood sample was obtained (e.g., mid-luteal phase). These blood samples were analyzed during a single laboratory session for estradiol (E_2), progesterone (P_4), luteinizing hormone (LH), follicle stimulating hormone (FSH), and sex hormone binding globulin (SHBG).

All subjects ingesting oral contraceptives (ORAL) were asked to report the exact preparation, duration of use and compliance to therapy. All oral preparations were of the ethinyl estradiol type (over 25 commercial products exist). Women ingesting preparations that included mestranol were excluded from the study. Subjects were asked to provide empty pill packs to the investigators to document therapy information. During the cycle immediately preceding training, and during the two months of training, all ORAL users had blood sampled during day 2, 3, 4, or 5 of the placebo phase to document compliance to therapy. These blood samples were analyzed for E_2, P_4, and SHBG.

All subjects receiving long-acting Depo-Provera (DP) contraceptive therapy were asked to report the exact dose, preparation, and duration of use. During the cycle immediately preceding training, and during the two months of training, all injectable DP users had blood sampled during days 2, 3, 4, or 5 of a given 28 day period (initiated on an arbitrary day) to document therapy. These blood samples were analyzed for medroxyprogesterone acetate (i.e., provided in the contraceptive DP), E_2, P_4, and SHBG.

BODY COMPOSITION AND MAXIMAL OXYGEN UPTAKE MEASUREMENTS

Body composition analyses were performed during the first seven days of each menstrual cycle or 28 day period, and at the beginning and end of the training period. Body density was determined from underwater weighing. Percent body fat and lean body mass were calculated according to Siri (56). Further, all subjects completed an incremental run to exhaustion (modification of Costill and Fox protocol) on a motorized treadmill for determination of VO_2 max (17). These tests were performed during the intensive screening period and following the exercise training program. Briefly, subjects ran at an appropriate speed for four minutes at 0% grade. After four minutes, the treadmill grade was increased to 4% for two minutes. The grade was then be increased 2% every two minutes until the subject reached volitional exhaustion. Two of the three following criteria were used to verify the attainment of VO_2 max: 1) an increase in VO_2 of less than 150 $ml \cdot min^{-1}$ with an increase in treadmill grade, 2) heart rate greater than 90% of predicted maximum (220 minus age), and 3) respiratory exchange ratio greater than 1.1.

EXERCISE-HEAT TOLERANCE TESTING

Exercise-heat tolerance (EHT) tests were performed at the beginning and end of the 8-week training program. To enhance the stress associated with the EHT, subjects undertook a 24-hour water restriction prior to testing, providing an approximate -3% level of dehydration. The EHT involved walking on a motorized treadmill at 93.6 $m \cdot min^{-1}$ and 5% grade (4). Walking speed was verified for each test with a hand-held tachometer (Model 8240-20 Cole Parmer Instrument Co., Chicago, IL). The mean temperature and relative humidity were 38°C and 50-70%, respectively. Air flow was 2.3 $m \cdot s^{-1}$. No water was consumed during the EHT. The test was terminated if: a) T_{re} reached 39.5°C, b) the heart rate exceeded 180 beats min^{-1} for five consecutive minutes, c) the subject showed signs of heat illness, d) the subject asks to stop, or e) she completed 90 minutes of exercise.

A schematic representation of events during each EHT test appears as **Figure 1**. The following physiological and perceptual measures were taken at regular intervals before, during and after EHT testing: oxygen uptake, minute ventilation, and respiratory exchange ratio using an on-line system (Medical Graphics Corporation); whole-body sweat rate (± 50 g) via body weight differences; mean weighted skin temperature (4 sites) via infrared temperature scanner (Ototemp, Inc.); subjective ratings of perceived exertion (10); and thermal stress (64). Rectal temperature (rectal thermistor, YSI Inc.), heart rate via cardiotachometer (Polar Electro), and exercise time were the primary variables representing exercise-heat tolerance. Measurements of local chest sweat rate using resistance hygrometry (Model B1-102, Bi-Tronics, Inc.) and local skin blood flow via laser doppler flowmeter (Techtronics, Inc) began in Year II and continued through Year III (see section below titled, "Problems Encountered in Accomplishing Tasks").

EHT TESTING: MENSTRUAL PHASE AND CONTRACEPTIVE THERAPY

All eumenorrheic ovulatory women were tested during day 2, 3, 4, or 5 of their menstrual cycle (i.e., early follicular phase). All oral contraceptive users were tested on day 2, 3, 4, or 5 of the 7 day placebo period for their respective pill packs. All Depo-Provera users were tested on day 2, 3, 4, or 5 of a 28 day period arbitrarily initiated during the preliminary screening period. The specific day on which testing occurs remained consistent for each subject.

EXERCISE TRAINING PLUS HEAT ACCLIMATION

The exercise training program lasted 7-8 weeks--two menstrual cycles--in duration. It was necessary to admit subjects into the training program in a staggered fashion to account for timing differences in menstrual cycle phase and contraceptive therapy. Training sessions were held six days per week (Monday - Saturday). Outdoor training sessions on Tuesday, Thursday, and Saturday involved strenuous running and calisthenics (push-ups and sit-ups) in a group, with a progressive increase in volume and speed of running across weeks that was encouraged verbally. The number of push-ups and sit-ups also progressively increased for eight weeks. All of these outdoor training sessions were supervised.

Indoor training sessions on Monday, Wednesday, and Friday also were supervised, and involved exercise-heat exposures (mean conditions inside the environmental chamber ranged from 36.0 ± 1.4 to 37.0 ± 1.5°C and 27.5 ± 5.3 to 33.2 ± 1.5 %rh) progressing toward 90 minutes of continuous exercise-heat acclimation each day. These sessions entailed 3-6 subjects exercising at one time, employing a circuit of bench stepping, cycle ergometry, and treadmill walking. Subjects were permitted to drink water ad libitum during these sessions. Subjects were encouraged to exercise continuously as long as possible during these sessions, but were asked to remain in the chamber for the complete 90 minute period,even if they stop exercising. However, subjects were removed from the environmental chamber if: a) T_{re} reached 39.5°C, b) heart rate exceeded 180 beats·min^{-1} for 5 consecutive minutes, or c) the subject showed signs of heat illness. . All of these indoor training sessions were supervised.

RESTING BLOOD COLLECTIONS: HORMONE & IMMUNE SYSTEM ANALYSES

Figure 2 presents the timeline for resting hormone and immunological analyses during the preliminary screening, the intensive screening, and the 8-week heat acclimation/training program. Blood samples were obtained by needle and syringe or indwelling cannula, collected into serum or plasma collection tubes and then processed, centrifuged, stored when appropriate at -80°C, and analyzed.

Concerning reproductive hormones and aldosterone, these blood samples allowed two baseline measurements (with respect to menstrual phase) prior to the start of the training protocol. Regarding the immune factor measures, two baseline measurements of each blood variable also were made prior to the start of training.

EHT BLOOD COLLECTIONS: HORMONE AND IMMUNE SYSTEM ANALYSES

The EHT tests were conducted before and after the 8-week training program, and on the day following the resting blood collections described above. Pre-exercise and immediate post-exercise blood samples were obtained via indwelling cannula and analyzed for whole blood, plasma, or serum concentrations of cortisol, epinephrine, norepinephrine, lactate, glucose, osmolality, hematocrit, hemoglobin, anti-LPS, and IgG. Analyses of anti-LPS and Total IgG also were performed on 24-hour and 48-hour post-exercise blood samples

REPRODUCTIVE HORMONE AND IMMUNE SYSTEM MEASUREMENTS

Serum E_2, P_4, prolactin, TSH, FSH, LH, thyroxin and SHBG were analyzed in the Department of Fertility and Reproductive Endocrinology at New Britain General Hospital via chemiluninescense immunoassay (Immulite^{R}). This procedure provides excellent sensitivity and reliability by combining highly specific antibodies with enzyme-amplified chemiluminescent chemistry and a proprietary wash technique. Serum aldosterone (19) and cortisol (33) concentrations were analyzed in the Human Performance Laboratory (University of Connecticut) via radioimmunoassay.

CD4 and CD8 determinations were performed on unseparated cells in whole peripheral blood, at the U.S. Army Research Institute of Environmental Medicine (USARIEM), Natick, MA. Briefly, whole heparinized blood was incubated with the specific fluorescent-labeled antibody. Red blood cells were lysed, the white cells fixed with 1% paraformaldehyde and the samples analyzed by flow cytometry.

[This section is continued on page 17]

Abbreviations Used in Figures 1 and 2

Figure 1

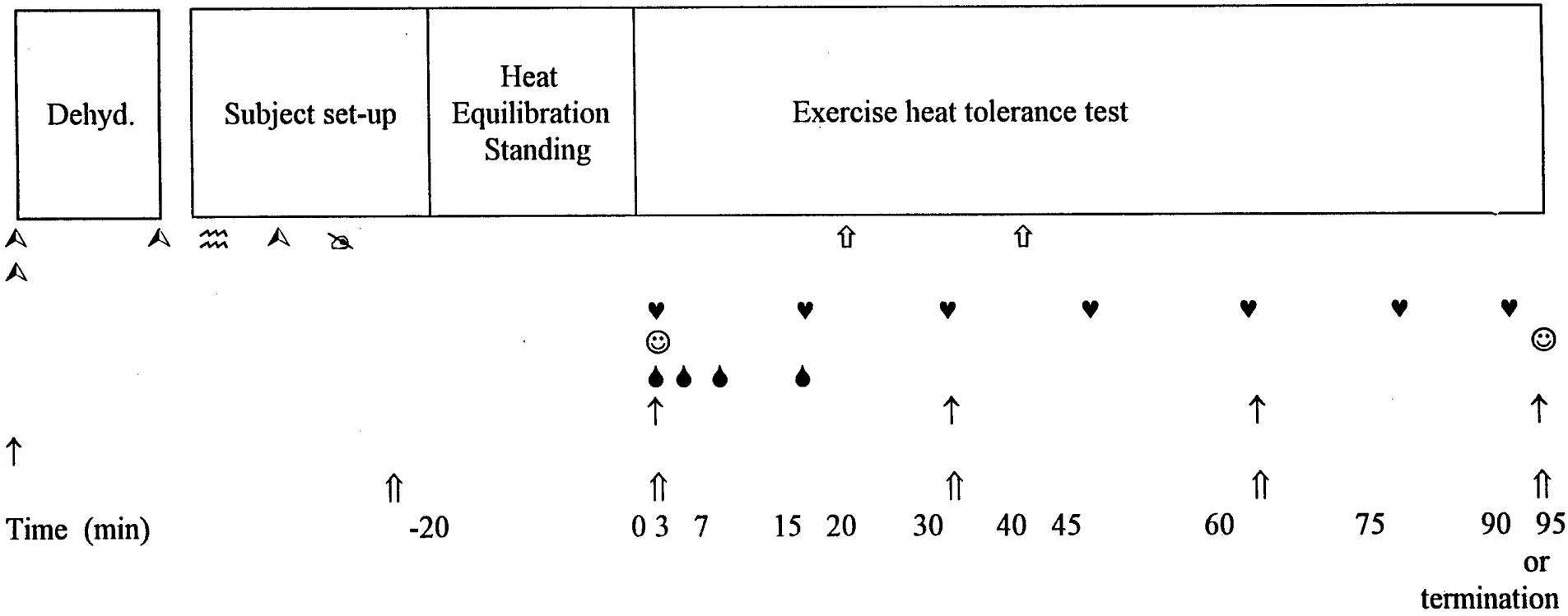
Dehyd. = dehydration to -3 % of body weight
HTT (also EHT) = the exercise-heat tolerance test conducted in our environmental chamber (38°C, 50-70% rh)
V_E = minute ventilation (L/min)
VO_2 = oxygen consumption
RER = respiratory exchange ratio (CO_2/O_2)
BP = blood pressure (systolic/diastolic)
MWST = mean weighted skin temperature, taken at four sites
T_{re} = rectal temperature

Figure 2

ALD = aldosterone
CD_x = cluster of differentiation (x = 4,8,14,45)
DP = Depo-Provera subjects
E_2 = estradiol
EE = ethinyl estradiol
EU-OV = eumenorrheic ovulatory subjects
F = follicular phase
FSH = follicle stimulating hormone
HSP_{70} = heat shock protein
IFN-g = interferon N
IG-1 = immunoglobulin 1
IL-x = interleukin (x = 6,10)
L = luteal phase
LH = luteinizing hormone
MPA = medroxyprogesterone acetate
ORAL = oral contraceptive subjects
P_4 = progesterone
PRL = prolactin
SHBG = sex hormone binding globulin
T_4 = thyroxin
TSH = thyroid stimulating hormone

FIGURE 1

Heat Tolerance Test Schematic



Measurements

- ≈ - void bladder and bowel; weigh exercise clothes prior to dressing and following final body weight
- ⊘ - rectal probe and cannula (insert prior to pre HTT & remove prior to post body weight)
- ☺ - blood draw @ following 20 min standing heat equilibration and at termination
- ⇧ - VO_2, V_E, RER @ min 20 & 40
- ♦ - Local chest sweat rate (Dew point sensor) @ min 0, 3, 7 & 15
- ♥ - HR, T_{re}, RPE, Thermal stress, @ min 0, every 15 min & at 89 min or at termination
- ⩓ - Body weight @ pre & post dehydration, pre & post HTT
- ↑ - BP @ min 0, 30, 60 & 90 or temination & 5 min post
- ⇑ - Skin blood flow & MWST (OtoTemp) @ min -25, 0, 30, 60, & 90 or termination

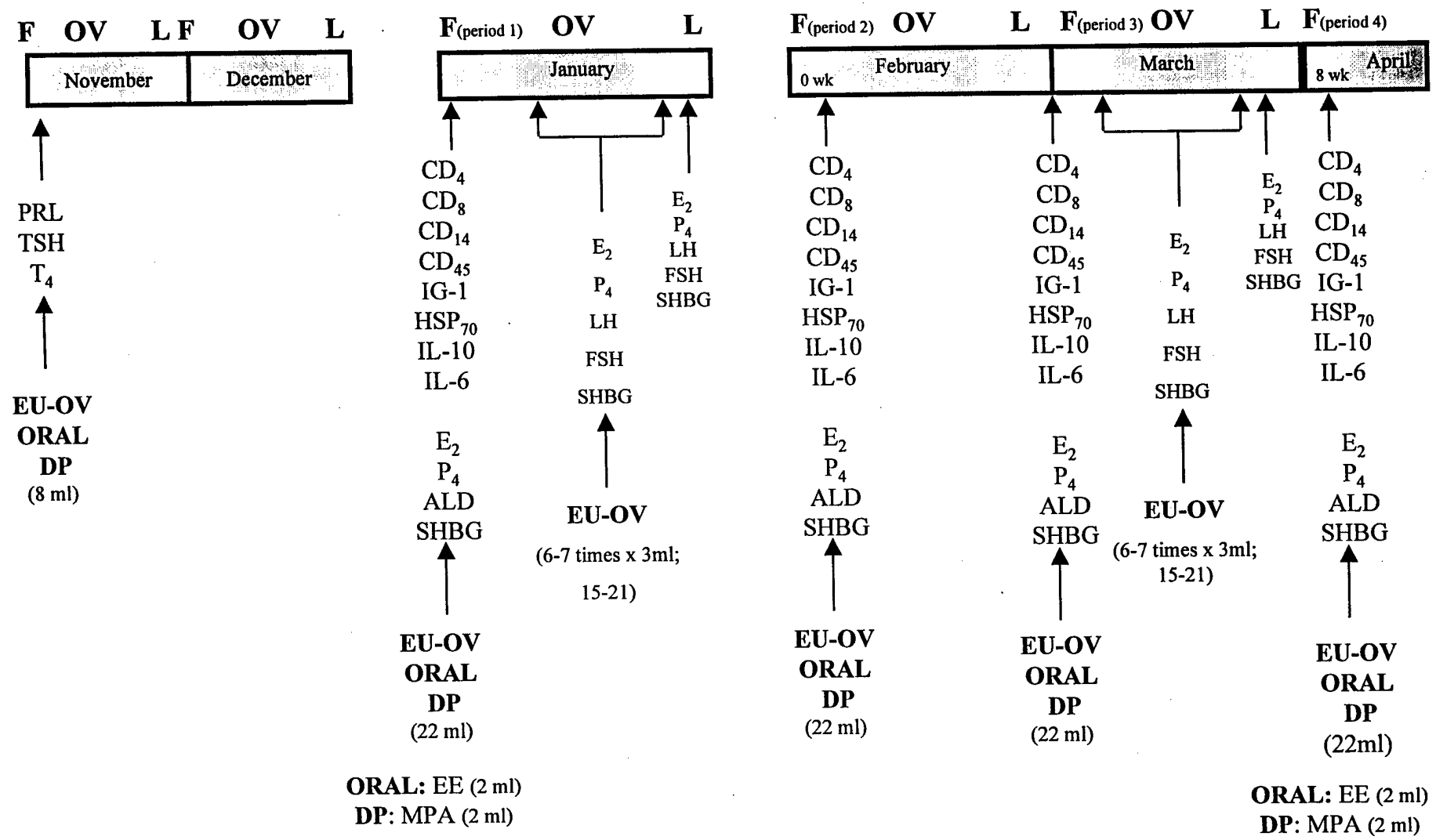


FIGURE 2: Resting blood collection for hormone and immune system measures. (Volumes of blood samples appear at each time point).

IL-10, TNF, and INF-g were measured at USARIEM in plasma by commercially available ELISA kits, during Year I only (see section below titled, "Problems Encountered in Accomplishing Tasks"). CD-14 and CD-45 were also measured, but only to provide data necessary to properly determine CD-4 and CD-8. Anti-LPS IgG as measured by an ELISA produced in-house, and Total IgG by an automated immunoenzyme system (Monarch) at USARIEM (26). We attempted attempt to measure HSP_{70} via either Western blot or PCR technology at USARIEM in Years II and III. Plasma norepinephrine and epinephrine levels were analyzed via an HPLC technique (33).

Hematocrit was determined by microcapillary technique. Hemoglobin was measured by the cyanmethemoglobin method (Kit 525, Sigma Chemical, Inc.) Percent change in plasma volume was calculated using hematocrit and hemoglobin values (20). Osmolality was measured by freezing point depression (model 5004 micro-osmette, Precision Systems, Inc.). Plasma lactate and glucose values were determined using a model 2300 glucose and L-lactate analyzer (Yellow Springs Instruments).

STATISTICAL METHODS

The final data base was analyzed via PC, using the CSS:Statistica™[3.1], Statsoft Data Management System. We used common descriptive statistics to describe the data sets. In addition, both univariate and multivariate statistics were used to determine group differences, main effects, interactions and relationships between variables. Appropriate post hoc tests were performed where significant F ratios were found. An alpha level of 0.05 was used to detect significant differences, between groups and across time.

Results and Discussion

DATA PRESENTATION

During Year I testing, 12 subjects were tested. During Year II, 11 subjects completed all phases of testing, but one of these was noncompliant and her data were eliminated from the database. During Year III, an additional 14 subjects participated. This brought the three-year total number of subjects to 36 (i.e., EU-OV, 14; ORAL, 15; DP, 7). Analyses of variance were performed to test between-group differences in this Final Report.

The entering personal characteristics of these 36 female participants were as follows (mean ± SD): age, 21 ± 3 yr; height, 64 ± 3 cm; body mass, 65.78 ± 11.11 kg; maximal aerobic power, 37.1 ± 4.1 ml/kg/min.

No serious illnesses, serious injuries, or untoward events occurred during Year I, Year II or Year III of testing.

REPRODUCTIVE HORMONES: SCREENING & EFFECTS OF 8 WEEKS OF TRAINING

The 36 women who participated in the study represented eumenorrheic-ovulatory women (n = 14, control group), oral contraceptive users (n = 15) and Depo-Provera users (n = 7). All subjects passed a hormonal assessment during the screening process. The initial assessment consisted of normal baseline TSH, free T_4, and prolactin.

Data for the eumenorrheic group are presented in terms of menstrual cycle milestones, while data for the oral contraceptive group are presented in terms of hormonal assessment, measured during the placebo week of each subject's pill pack. Subjects in the eumenorrheic group were assessed for one complete menstrual cycle; this assessment consisted of E_2, LH, FSH, SHBG, prolactin and P_4 measurements. During the menstrual cycle assessments, blood was drawn during the early follicular phase (days 2-6), the mid-to-late follicular phase (days 8-19), and the mid-luteal phase (5-7 days following the LH peak). Cycle length, ovulation day, normal luteal phase, normal hormonal characteristics and previous cycle lengths were utilized as criteria for admission to the study. Once admitted to the study, eumenorrheic subjects underwent a second complete menstrual cycle assessment during the second month of the 8-week training program.

The oral contraceptive group was evaluated for these same hormones, during the training sessions and following the 8 week training program. Hormonal assessments (estradiol, progesterone, prolactin, SHBG) of oral contraceptive users were done during the placebo phase of their pill regimen.

Table 1 (mean ± SE) shows that, overall, the three experimental groups responded to the physical training-heat acclimation regimen unremarkably during this investigation:

- The ovulatory status of the EU-OV group at post-training was similar to the pre-training period. Thus, the exercise training accomplished by these women had no significant effect on menstrual status in this study. Menstrual cycle length was unchanged and unremarkable. However,
  a) one of the Year III participants had one anovulatory cycle, of unknown etiology and
  b) the mean post-training prolactin level of EU-OV subjects was higher ($P < .05$) than their pre-training level.
- Hormonal responses of the EU-OV and both contraceptive groups are similarly unremarkable.
- As expected, the ORAL group had a greater ($P < .05$) serum SHBG level than the EU-OV and DEPO groups, at both pre-training and post-training time points.
- Two reproductive characteristics in the EU-OV group are of particular interest:
  a) the follicular and luteal phases were unchanged, and
  b) peak serum progesterone was unchanged.

Therefore, these data indicate that the multiple stressors provided by eight weeks of physical training and heat exposure had no important physiological effects on either menstrual or hormonal status.

PHYSICAL TRAINING AND HEAT ACCLIMATION (8 WEEKS)

Three days of each week were spent performing stretching, calisthenics (i.e., pushups and situps), and walking/running a 2.85-mi course around campus. The remaining three training days were spent in the Human Performance Laboratory's environmental chamber, performing various types of exercise (i.e., cycling, treadmill walking, bench stepping) in conditions of 37°C, 30-50 %rh; these sessions were designed to induce heat acclimation in all subjects. During exercise-heat acclimation sessions, subjects exercised 72-88 min out of the total 90 min heat exposure. Subjects did not train on Sunday of each week. The proposed 8-week training schedule was accomplished with a very high compliance rate for daily exercise sessions and heat exposures (> 95%, including minor illnesses).

The following measurements were made to track the progress of the physical training of test subjects, including:

-- number of pushups and situps (i.e., abdominal crunches) completed in 1 min
-- time to complete the 2.85 mi outdoor course
-- body composition changes (i.e., body mass, % body fat, fat-free mass)
-- maximal aerobic power (VO_{2max}).

The physical training criterion variables appear in **Table 2** below, as recorded during the initial and final weeks of this training program. Values reflect group means ± SD. Column four indicates that all values except body mass were significantly different (pre- versus post-training/acclimation), as assessed by analysis of variance (significant main effect: time). These measurements indicate that the three groups of test subjects were stronger, more physically fit, and leaner at the end of the 8-week physical training program. There were no significant differences between groups, for any variable in **Table 2**.

Table 1 - Reproductive Hormones and Menstrual Phase Lengths: Pre- and Post-Training

	Pre-Training	Post-Training
Eumenorrheic Group		
Early Follicular		
Estradiol	30.7±3.1	26.4±2.6
Progesterone	0.8±0.1	0.9±0.1
Prolactin	13.8±2.0	18.4±2.2[b]
SHBG	44.3±3.0	45.4±4.0
Mid-Cycle		
Peak estradiol	232.1±22.2	249.5±24.9
Peak LH	44.4±4.1	44.1±4.9
Mid-luteal		
Peak estradiol	131.6±15.7	132.9±11.0
Peak progesterone	12.9±1.4	12.5±0.9
Cycle Parameters		
Cycle length	27.1±0.6	28.1±1.0
Ovulation day	15.2±0.4	15.2±0.6
Follicular length	15.2±0.4	15.2±0.6
Luteal length	11.9±0.3	12.8±0.4

	Pre-Training	Post-Training
ORAL Contraceptive Group		
Estradiol	32.9±4.9	29.1±3.2
Progesterone	0.9±0.2	0.9±0.1
Prolactin	14.7±2.1	15.2±2.1
SHBG	157.1±14.5[a]	180.0±20.6[a]

	Pre-Training	Post-Training
DEPO Group		
Estradiol	23.9±2.3	22.4±1.6
Progesterone	0.8±0.2	0.8±0.1
Prolactin	18.3±5.2	22.8±8.8
SHBG	39.9±7.7	39.8±7.0

Abbreviations: LH - luteinizing hormone; SHBG - sex hormone binding globulin.
Values in this figure are expressed as mean ± SE.
Symbols: a - $P<0.05$, ORAL versus EU-OV and ORAL versus DEPO,
b - pre-training versus post-training (EU-OV only)

Table 2. Physical Training Variables

MEASUREMENT (units)	INITIAL WEEK *	FINAL WEEK	STAT. SIGNIF.
Pushups (per 60 sec)			
ORAL	19 ± 6	45 ± 9	
EU-OV	17± 10	44 ± 26	#
DEPO	20 ± 19	37 ± 17	
Situps (per 60 sec)			
ORAL	53 ± 10	76 ± 20	
EU-OV	49 ± 12	71 ± 21	#
DEPO	48 ± 10	75 ± 13	
2.8 mile run time (min)			
ORAL	40.1 ± 5.8	30.6 ± 6.8	
EU-OV	39.4 ± 7.9	28.6 ± 3.1	#
DEPO	39.2 ± 9.1	30.5 ± 5.4	
Body Mass (kg)			
ORAL	65.8 ± 12.4	65.5 ± 11.6	
EU-OV	62.8 ± 9.4	63.2 ± 8.8	
DEPO	71.6 ± 10.5	72.1 ± 11.1	
Body Fat (%) **			
ORAL	28.0 ± 5.1	26.0 ± 5.0	
EU-OV	26.5 ± 5.9	24.6 ± 5.9	#
DEPO	31.8 ± 5.6	30.4 ± 6.0	
Fat Free Mass (kg) **			
ORAL	46.7 ± 5.7	47.9 ± 5.4	
EU-OV	45.7 ± 4.5	47.3 ± 4.7	#
DEPO	46.4 ± 3.1	47.8 ± 3.9	
$VO_{2\,max}$ ($l \cdot min^{-1}$)			
ORAL	2.43 ± 0.27	2.69 ± 0.25	
EU-OV	2.38 ± 0.31	2.62 ± 0.26	#
DEPO	2.55 ± 0.32	2.73 ± 0.30	
$VO_{2\,max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)			
ORAL	37.1 ± 3.6	41.5 ± 4.1	
EU-OV	37.7 ± 3.8	41.4 ± 2.8	#
DEPO	35.7 ± 5.6	38.1 ± 6.1	

All Data are reported as mean ±SD. Abbreviations: $VO_{2\,max}$ – maximal aerobic power. Symbols: * – pre-training; ** – derived from hydrostatic weighing;; # = $P < 0.05$, Main effect:TIME (for all groups); no between-group differences existed

EXERCISE-HEAT TOLERANCE (EHT) TESTING

Specialized EHT tests were administered to each subject, before and after the 8-week training period. EHT tests consisted of walking on a motorized treadmill at 93.6 $m \cdot min^{-1}$ and 5% grade. The conditions inside the environmental chamber ranged from 36.0 $\pm$ 1.4 to 37.0 $\pm$ 1.5°C and 27.5 $\pm$ 5.3 to 33.2 $\pm$ 1.5 %rh,with a constant air flow of 2.3 $m \cdot s^{-1}$. To enhance the stress associated with the EHT, subjects underwent exercise combined with a period of 12-hour water restriction prior to testing, providing a dehydration of -2.8 $\pm$ 0.6 % before training and -2.9 $\pm$ 0.6 % after eight weeks of training. No water was consumed during the EHT.

The values recorded during the pre-training and post-training EHT tests appear below in **Table 3**, expressed as group means ± SD. Column 4 indicates which values were significantly different across time (pre- versus post-acclimation), as assessed by analysis of variance (significant mean effect: time).

Each of the measurements in **Table 3** indicate that the three groups of test subjects achieved heat acclimation at the end of the 8-week training program. Final heart rate, rectal temperature, skin temperature, and rating of perceived exertion values were lower after eight weeks of exercise-heat exposure (6 days/week). Interestingly, the whole-body sweat rate did not increase after heat acclimation, probably because the rectal temperature was lower during post-acclimation testing, thereby stimulating a lower efferent sweating response at the skin.

There were no significant differences *between groups*, for any of the variables (pre- versus post-acclimation) in **Table 3** except that the whole-body sweat rate tended to be higher in DEPO (versus ORAL and EU-OV).

Skin blood flow was measured with the laser doppler flowmeter, at a site on each subject's forearm. When considered with local sweat rate (below), this data can be used to evaluate heat dissipation in each experimental group. **Table 4** presents the results of skin blood flow measurements. Values represent the percent change of skin blood flow (SBF), from a resting baseline condition (cool 23°C environment) to an exercise-induced state (hot 37°C environment). For example, SBF during the pre-acclimation EHT for the ORAL group was 69.1% greater during exercise than at baseline. Baseline SBF was performed dehydrated, prior to the EHT, in a 23°C ambient temperature. Exercise SBF was performed at the 15 min point of the EHT, in 37°C ambient temperature. There were no significant differences in this variable, between groups or across time.

Table 3. Exercise-Heat Tolerance Test (EHT) Physiological Variables

MEASUREMENT (units)	PRE-ACCLIMATION	POST-ACCLIMATION	STAT. SIGNIF.
Pre-exercise dehydration (% loss)			
ORAL	-2.7 ± 0.5	-2.9 ± 0.6	
EU-OV	-3.0 ± 0.6	-3.0 ± 0.5	
DEPO	-2.7 ± 0.5	-2.7 ± 0.6	
Exercise time to exhaustion (min) *			
ORAL	45.6 ± 16.1	76.0 ± 19.2	
EU-OV	45.6 ± 19.3	75.3 ± 12.8	#
DEPO	34.0 ± 13.0	67.1 ± 15.2	
Final heart rate (beats/min) **			
ORAL	178 ± 12	155 ± 14	
EU-OV	179 ± 10	155 ± 13	#
DEPO	186 ± 9	156 ± 12	
Final rectal temperature (°C) **			
ORAL	38.5 ± 0.3	38.1 ± 0.2	
EU-OV	38.6 ± 0.4	38.2 ± 0.5	#
DEPO	38.4 ± 0.6	38.2 ± 0.2	
Final mean skin temperature (°C) **			
ORAL	35.5 ± 0.8	34.5 ± 0.9	
EU-OV	35.6 ± 0.5	34.3 ± 0.9	#
DEPO	34.7 ± 1.1	34.0 ± 0.9	
Final rating of perceived exertion **			
ORAL	18 ± 2	14 ± 2	
EU-OV	17 ± 2	14 ± 2	#
DEPO	17 ± 3	13 ± 1	
Final whole-body sweat rate (L/h)			
ORAL	0.72 ± 0.25	0.75 ± 0.12	
EU-OV	0.91 ± 0.35	0.67 ± 0.21	##
DEPO	1.03 ± 0.20	0.99 ± 0.26	

All data are reported as mean ±SD. Abbreviations: $VO_{2\,max}$ – maximal aerobic power. Symbols: * – or reaching prescribed safety limits of rectal temperature, heart rate, etc.; ** – at same time point in both tests (i.e., the endpoint of the pre-acclimation EHT); # = $P<0.05$, main effect: time (all groups combined); no between-group differences existed; ## = $P<0.05$, main effect: between-group difference (DEPO versus ORAL and DEPO versus EU-OV)

Table 4. Exercise-Induced Skin Blood Flow Change During the Exercise-Heat Tolerance Test (% Change from Baseline)

MEASUREMENT	PRE-ACCLIMATION	POST-ACCLIMATION	SIGNIFICANCE
ORAL	69.1 ± 89.5	48.3 ± 59.1	--
EU-OV	81.3 ± 81.9	104.8 ± 93.3	--
DEPO	92.4 ± 85.2	30.6 ± 34.9	--

All data are presented as means ±SD.

LOCAL SWEATING RESPONSES

Local sweat rate was measured with a dew point sensor, placed on each subject's back, before and after the 8-week training/acclimation period. Measurements were made in a 23 °C ambient temperature, while subjects exercised on a cycle ergometer. Their body water was normal (euhydrated). Two variables were measured: the threshold temperature for the onset of sweating, and the slope of the line representing the relationship between rectal temperature and local sweat rate (e.g., sweat sensitivity). **Table 5** presents these two variables, as measured before and after the 8-week training/acclimation period.

Figure 3 depicts the relationship between rectal temperature and local sweat rate in all groups, as measured before and after the 8-week acclimation/training period. There were no significant between-group findings. The **threshold temperature** for the onset of sweating decreased ($P<0.05$) in the ORAL group only. The **sweat sensitivity (slope)** of this relationship was unchanged in all groups.

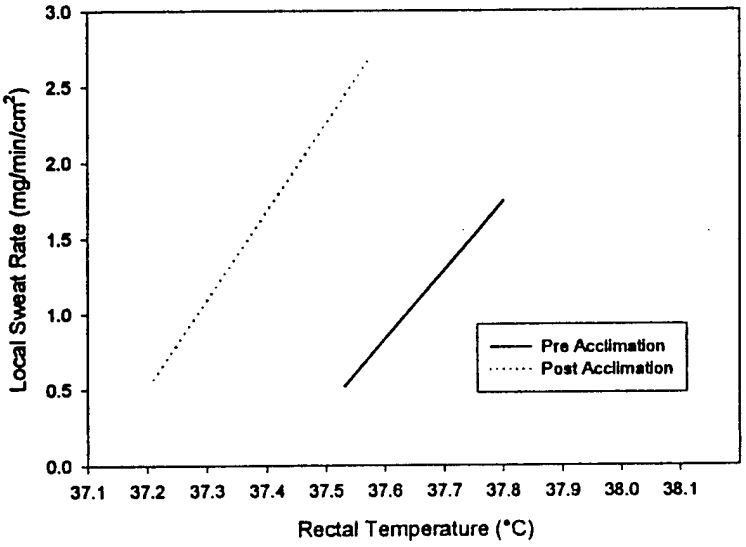
Table 5. Local Sweating: Pre- vs. Post-Acclimation

MEASUREMENT	PRE-ACCLIMATION		POST-ACCLIMATION
Threshold Rectal Temperature for the Onset of Sweating (°C)			
ORAL	37.5 ± 0.2	a	37.2 ± 0.4
EU-OV	37.5 ± 0.2		37.4 ± 0.2
DEPO	37.7 ± 0.1	b	37.7 ± 0.2
Sweat Sensitivity (slope, see Fig. 3)			
ORAL	4.0 ± 2.2		5.3 ± 3.2
EU-OV	3.8 ± 3.5		3.5 ± 2.2
DEPO	2.5 ± 1.0		3.7 ± 2.1

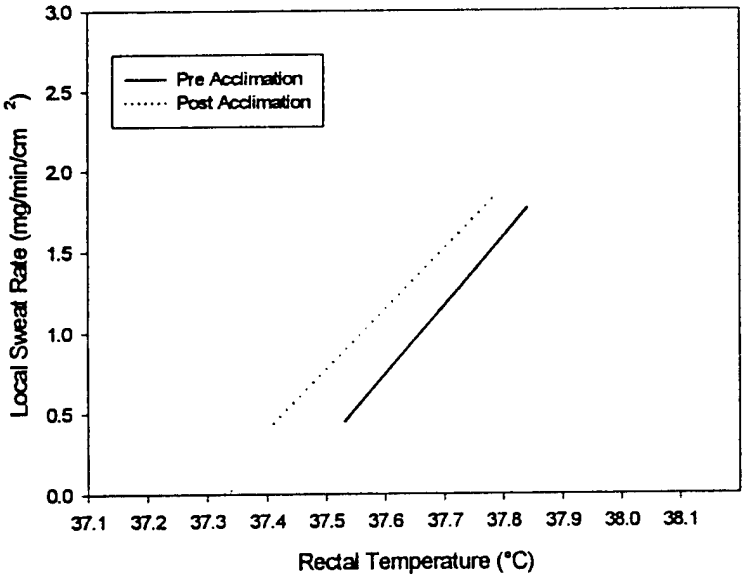
All data are presented as mean ±SD.
Symbols: a = significant difference across time (ORAL group only);
b = $P<0.05$, significant difference between DEPO and ORAL (during the post-acclimation test only)

Figure 3

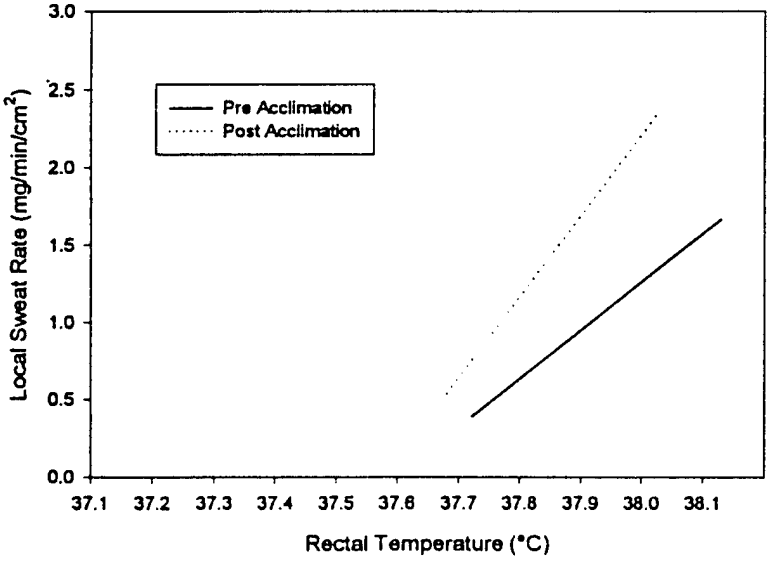
ORAL



EU-OV



DEPO



EXERCISE-HEAT TOLERANCE (EHT) TESTING (cont.)

Table 6 contains values for the following variables, measured in blood during the EHT tests (pre- and post-training): the percent change in plasma volume (%CHGPV), glucose, lactate, osmolality, cortisol, epinephrine, and norepinephrine. Values from pre-EHT (pre-exercise) and post-EHT (post-exercise) blood samples are presented. Plasma epinephrine and norepinephrine measurements were performed at the Core Endocrine Laboratory of the Hershey Medical Center, Hershey, PA, under the direction of Dr. Lawrence Demers. All other values were determined at the Human Performance Laboratory, University of Connecticut.

The results in **Table 6** demonstrate that 8 weeks of exercise training and heat acclimation had significant effects ($P<0.05$) on some plasma variables. These differences are described below.

%CHGPV: No differences were observed between groups or across time.

Plasma Glucose: No differences were observed between groups or across time.

Plasma Lactate: Post-exercise (but not pre-exercise) lactate levels were lower after eight weeks of physical training and heat acclimation.

Plasma Osmolality: Pre-exercise (but not post-exercise) plasma osmolality values were lower after eight weeks of exercise-heat exposure.

Plasma Cortisol: The cortisol levels of the ORAL group were higher than both the EU-OV and DEPO groups, at all four time points.

Plasma Epinephrine: No differences were observed between groups or across time.

Plasma Norepinephrine: No between-group differences existed. Pre-exercise (PRE-EHT) levels of norepinephrine were lower, after eight weeks of exercise-heat exposure. Within the pre-acclimation EHT test only, the concentration increased significantly from pre-exercise to post-exercise.

RESTING ALDOSTERONE LEVELS IN BLOOD

Aldosterone levels were measured at rest, in early morning blood samples, at three time points: before (PRE), at 4 weeks (MID), and at 8 weeks of physical training (POST). **Table 7** demonstrates that there were no statistically significant differences in circulating aldosterone levels, either between the ORAL and EU-OV groups or across time in either group.

IMMUNOLOGICAL FACTORS

The analyses performed during Year I through Year III were as follows:

1. Lymphocyte phenotypes of CD4+ (measure of T_{helper} cells) and CD8+ (measure of $T_{suppressor/cytotoxic}$ cells) by Flow Cytometry
2. Anti-Lipopolysaccharide Immunoglobulins (Anti-LPS) by ELISA
3. Total IgG by Monarch immunoassay.

All of the above analyses were performed on EDTA-preserved blood samples that were collected, kept in an insulated container at room temperature and either transported immediately to USARIEM for processing for flow cytometric studies, or processed into plasma and stored at -80°C until all samples were collected and then brought frozen to USARIEM for determination of Anti-LPS, Total IgG and Cytokines. Interleukin-10, interferon gamma, and tumor necrosis factor-alpha analyses were not performed in Years II and III because of technical/training/ manpower difficulties (see section below titled, "Problems Encountered in Accomplishing Tasks", item #3). Partial data for IFNg, TNF, and IL-10 appeared in our previous Annual Reports for 1996-97 and 1997-98.

[This section is continued on page 27]

Table 6. EHT Blood Variables

MEASUREMENT (units)	PRE-ACCLIMATION		POST-ACCLIMATION		STAT.
	PRE-EHT	POST-EHT	PRE-EHT	POST-EHT	SIGNIF.
%CHGPV (%)					
ORAL		-2.4 ± 3.5		-3.8 ± 4.4	
EU-OV		-3.9 ± 4.9		-4.7 ± 7.0	
DEPO		-1.2 ± 4.2		-3.3 ± 3.9	
plasma glucose (mg/dl)					
ORAL	96.9 ± 11.8	107.3 ± 16.6	96.7 ± 12.0	108.3 ± 21.6	
EU-OV	100.6 ± 16.3	101.4 ± 8.9	98.1 ± 12.3	111.7 ± 15.7	
DEPO	98.2 ± 12.2	115.7 ± 15.6	97.1 ± 11.1	115.8 ± 26.9	
CV = 1.0%					
plasma lactate (mmol)					
ORAL	1.3 ± 0.4	1.8 ± 0.7	1.3 ± 0.4	1.5 ± 0.4	*
EU-OV	1.3 ± 0.5	2.0 ± 0.3	1.1 ± 0.5	1.8 ± 0.7	
DEPO	1.1 ± 0.2	2.1 ± 0.3	1.0 ± 0.2	1.5 ± 0.7	
CV = 1.5%					
plasma osmolality (mosmol/l)					
ORAL	290.7 ± 3.7	296.0 ± 4.2	288.4 ± 4.3	297.4 ± 4.8	
EU-OV	291.1 ± 3.9	296.0 ± 4.4	288.9 ± 3.3	296.8 ± 4.4	†
DEPO	290.1 ± 4.2	296.2 ± 4.8	286.6 ± 2.2	296.2 ± 3.5	
CV = 0.2%					
plasma cortisol (nmol/l)					
ORAL	884 ± 197	825 ± 256	898 ± 268	1005 ± 432	
EU-OV	565 ± 252	606 ± 264	469 ± 194	722 ± 245	Ψ
DEPO	433 ± 185	602 ± 214	398 ± 122	547 ± 169	
CV = 7.8%					
plasma epinephrine (pg/ml)					
ORAL	81 ± 110	51 ± 48	56 ± 50	186 ± 278	
EU-OV	71 ± 68	147 ± 159	42 ± 22	109 ± 101	
DEPO	41 ± 27	42 ± 24	29 ± 12	33 ± 16	
CV = 15.0%					
plasma norepinephrine (pg/ml)					
ORAL	370 ± 136	1025 ± 573	222 ± 77	868 ± 347	# †
EU-OV	369 ± 144	1312 ± 512	342 ± 107	1187 ± 371	
DEPO	336 ± 82	1252 ± 421	316 ± 133	1173 ± 518	
CV = 9.0%					

CV - interassay coefficient of variation for that analysis; all runs for a given subject were completed within the same assay, to minimize the interassay variability.

* - $P<0.05$ time: Pre-Acclimation (POST-EHT) vs. Post Acclimation (POST-EHT)

† - $P<0.05$ time: Pre-Acclimation (PRE-EHT) vs. Post-Acclimation (PRE-EHT)

Ψ - $P<0.05$ between groups: ORAL versus EU-OV and ORAL versus DEPO, at all four time points.

- $P<0.05$ time: within Pre-Acclimation (PRE-EHT vs. POST-EHT)

Table 7. Morning Blood Levels of Aldosterone (at rest)

MEASUREMENT (units)	PRE	MID	POST
Aldosterone (pmol/l)			
ORAL	852 ± 357	745 ± 296	688 ± 271
EU-OV	639 ± 439	687 ± 377	517 ± 294
DEPO	594 ± 224	590 ± 358	843 ± 429
CV = 8.5%			

Abbreviations: PRE - before physical training began; MID - at 4 weeks of physical training; POST - after 8 weeks of physical training had been completed; CV - interassay coefficient of variation for that analysis; all runs for a given subject were completed within the same assay, to minimize the interassay variability.
No significant differences existed across time or between groups (ORAL versus EU-OV).

IMMUNOLOGICAL FACTORS (cont.)

The studies described in a- d below were conducted under a variety of conditions, some not properly controlled, on women of various ages, fitness, stages of their menstrual cycle, and contraceptive cycles so that it is difficult to relate their conclusions unambiguously to women soldiers.

a) Women versus Men. In general, due to the influence of sex hormones, particularly estrogens on T cells, B cells and phagocytes, non-pregnant females have more active humoral and cellular immune systems than males (65,66). Castration of males or administration of estrogen increases their immunity and resistance to infections (66,67). Females have higher serum immunoglobulin levels, greater antibody production in response to antigens, slower growth of tumors, and greater survival for melanoma (67 - 70). Because of their more active immune systems, they also have higher rates of autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and Hashimoto's thyroiditis) (65,66,69).

b) Pregnancy. During pregnancy, the elevated progesterone concentration stimulates the production of immunosuppressive factors by proliferative endometrial tissue (71). As a result, during pregnancy, cell-mediated immunity, mitogenic responses, and NK activity are depressed so that skin homografts survive longer than in non-pregnant women (67,72). This immunosuppression of pregnancy is important in preventing fetal rejection (66,73).

c) Contraceptives. While early studies indicated that oral contraceptives were immunosuppressive (74), no significant differences in antibody production or lymphocyte subsets were seen (in recent studies) between those taking modern low-dose combination oral contraceptives and those not taking them (75,76,77).

d) Estrogen and progesterone. Immune function in women varies with the stage of the menstrual cycle and is higher in the luteal phase than in the follicular phase (66). Estrogen analogs inhibit the elevated protein kinase C activity in activated lymphocytes (78). Estrogens, progesterone, and androgens influence host defenses by modulating phagocyte activity of macrophages, stimulating B cell differentiation, and enhancing antibody production (66,79 - 82). However, the role of estrogen on T cell subsets is controversial and may be related to the timing of the measurements . In some studies, estrogen treatment increased the number of CD4 cells, suggesting that estrogen influences T cell maturation (83). In other studies, no correlation was seen between estrogen, progesterone and the T cell subsets (84). In menopausal women receiving estradiol and oral medroxyprogesterone for six months, CD4 fell only after a week while CD8 fell after 3 weeks but then recovered (81).

Our Immunological Findings.

1. There was no significant effect of contraceptives on the concentrations of total IgGs or IgGs specific to lipopolysaccharides, or to the ratio of CD4:CD8 T lymphocytes in peripheral blood.

2. The Oral contraceptive group (compared to ORAL and DEPO) showed a small reduction of %CD8 lymphocytes at PRE- and POST- periods, whereas the Depo-group showed a small rise (compared to EU-OV, but not ORAL) in %CD4 lymphocytes.

3. The activity of the immune system is generally considered to be most closely related not to the % CD4 or %CD8 but to their ratio (21). The changes in %CD4 and %CD8 seen here were small, but there was no significant change in their ratio. Therefore, overall, the effect of oral contraceptives on the immune system as measured here is small or physiologically unimportant.
4. Limited studies on circulating cytokines (Year I) indicated no obvious trend.

Problems Encountered in Accomplishing Tasks

1. In Year III of this study, special efforts were undertaken to recruit Depo Provera users on the University of Connecticut campus and in the surrounding community. These efforts included posting of fliers specific to Depo-Provera users, identification of Depo-Provera users by the Student Health Services staff, recruiting at off-campus counseling centers, and specific recruiting by physicians in the surrounding community. As a result, a total of 7 DEPO subjects completed testing in all years. We learned that Depo Provera is not commonly prescribed by physicians, and is not a contraceptive of choice, for women at/near the University of Connecticut.

2. The timing of receipt of funding, ordering through the university procurement system, and delivery of two instruments (i.e., laser doppler and dew point sensor) made it impossible to use these instruments in Year I pre-training tests. Measurements of skin blood flow and local sweat rate were made in Year II and Year III only.

Due to the capacities and idiosyncrasies of these instruments, pilot studies (Fall, 1997) determined that it was better to measure the PRE-EHT skin blood flow in a cool environment (23°C, outside the environmental chamber) and to measure skin blood flow following 15 min of exercise in the heat (37°C, inside the environmental chamber). This allowed the change (delta) in skin blood flow to be calculated from rest to exercise. Other pilot studies (Summer, 1997) determined that the *onset temperature* and the *slope* of local sweating (i.e., the relationship between rectal temperature and sweat rate) were best measured during exercise in a cool/mild environment, because of saturation of the dew-point sensor capsule. These data (**Table 4, Table 5, Figure 3**) provide information regarding the effects of training and heat acclimation on heat dissipation and temperature regulation.

3. During 1997, the determinations of tumor necrosis factor-alpha (TNFa), interferon gamma (INFg), and interleukin-10 (IL-10) indicated that these cytokines displayed no specific trends or significant differences. Because cytokine assays are very expensive, analytical funds were severely limited, and because the relevance of TNFα as an index of immune status has been questioned, we decided to eliminate the TNFα, INFg and IL-10 analyses in subsequent years (J.L. Rossio. In: Committee on Military Nutrition Research. *Nutrition and Immune Function: Strategies for Sustainment in the Field.* Proceedings of a Workshop, Fort Detrick, MD, 1996. National Academy Press.).

5. In Year I, it was necessary to transport fresh blood samples on a regular basis to both New Britain General Hospital, New Britain, CT and U.S. Army Research Institute of Environmental Medicine, Natick, MA, because those samples could not be frozen and required timely analysis (i.e., within four hours of blood collection). Our original budget did not specify a line item for this travel expenditure, which involved nine different individuals using their privately-owned automobiles for this purpose. A review of the Project Year I budget revealed that approximately $3,000 was used for this purpose. We reported in the Year I Annual Report that it would be ideal to use a courier service for this purpose, to remove the burden and liability of such travel on research personnel. We utilized a courier service to ship blood samples during Year II, at a cost of $2,887.00. In Year III, our expense for this courier service was $2,718.75.

Abbreviations Used in Figures 4 - 6

ORAL - Oral Contraceptive (n = 15)
EU-OV - Eumenorrheic-Ovulatory (n = 14)
DEPO - Depo Provera (n = 7)

Figure 4

CD-4+ = cluster of differentiation #4; CD-8+ = cluster of differentiation #8
Pre = Baseline (pre-training) Mid = Mid-training (~ 4 weeks) Post = At end of 8 weeks of training

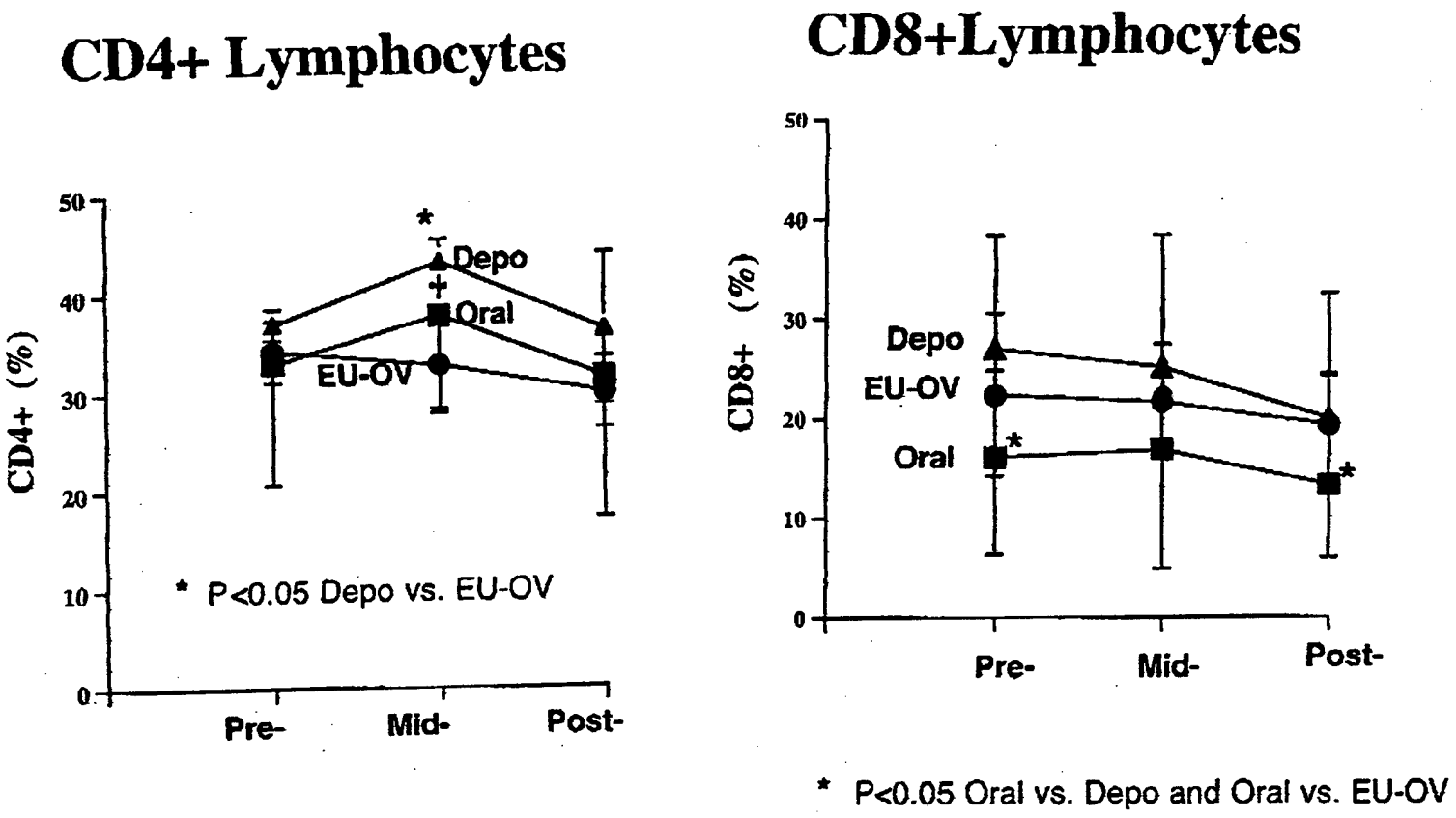
Figure 5

LPS = lipopolysaccharide (a toxic cell wall component of gram negative bacteria)
anti-LPS = the antibody formed in response to the circulating antigen LPS
Note: All values in this figure were collected during EHT #1 and #2. The dashed line between Period 2b and 3 represents the boundary between pre-training (Periods 1 - 2b) and post-training (Periods 3 - 4b).
Period 1 = Pre-training (before exercise began in EHT #1)
Period 2 = Pre-training (immediately following exercise in EHT #1)
Period 2a = Pre-training (24 h after the exercise of EHT #1)
Period 2b = Pre-training (48 h after the exercise of EHT #1)
Period 3 = Post-training (before exercise began in EHT #2)
Period 4 = Post-training (immediately following exercise in EHT #2)
Period 4a = Post-training (24 h after the exercise of EHT #2)
Period 4b = Post-training (48 h after the exercise of EHT #2)

Figure 6

IgG = immunoglobulin G. The dashed line between Period 2b and 3 represents the boundary between pre-training (Periods 1 - 2b) and post-training (Periods 3 - 4b).
Period 1 = Pre-training (before exercise began in EHT #1)
Period 2 = Pre-training (immediately following exercise in EHT #1)
Period 2a = Pre-training (24 h after the exercise of EHT #1)
Period 2b = Pre-training (48 h after the exercise of EHT #1)
Period 3 = Post-training (before exercise began in EHT #2)
Period 4 = Post-training (immediately following exercise in EHT #2)
Period 4a = Post-training (24 h after the exercise of EHT #2)
Period 4b = Post-training (48 h after the exercise of EHT #2)

Figure 4


CD4+ Lymphocytes
CD4+ (%)
50
40
30
20
10
0
*
Depo
Oral
EU-OV
* P<0.05 Depo vs. EU-OV
Pre-
Mid-
Post-
CD8+Lymphocytes
CD8+ (%)
50
40
30
20
10
0
Depo
EU-OV
Oral
*
*
Pre-
Mid-
Post-
* P<0.05 Oral vs. Depo and Oral vs. EU-OV

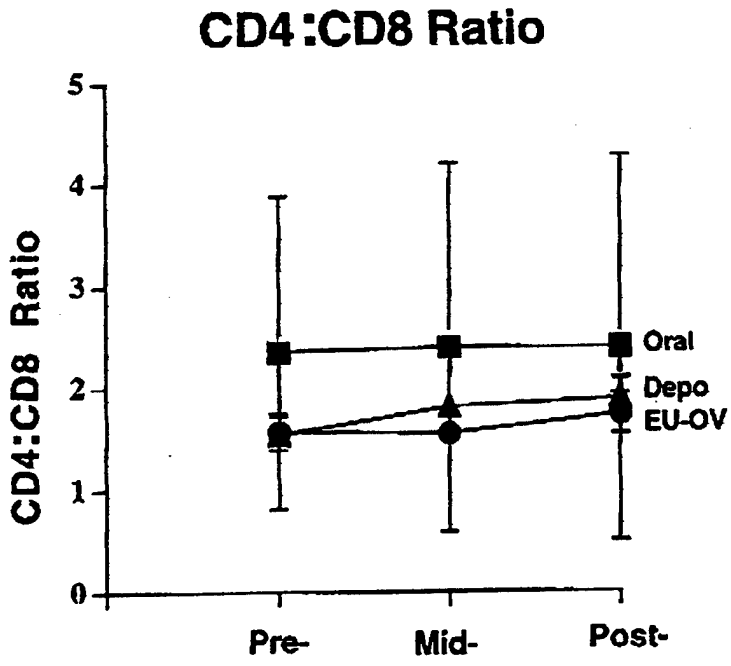

CD4:CD8 Ratio
CD4:CD8 Ratio
5
4
3
2
1
0
Oral
Depo
EU-OV
Pre-
Mid-
Post-

Figure 5

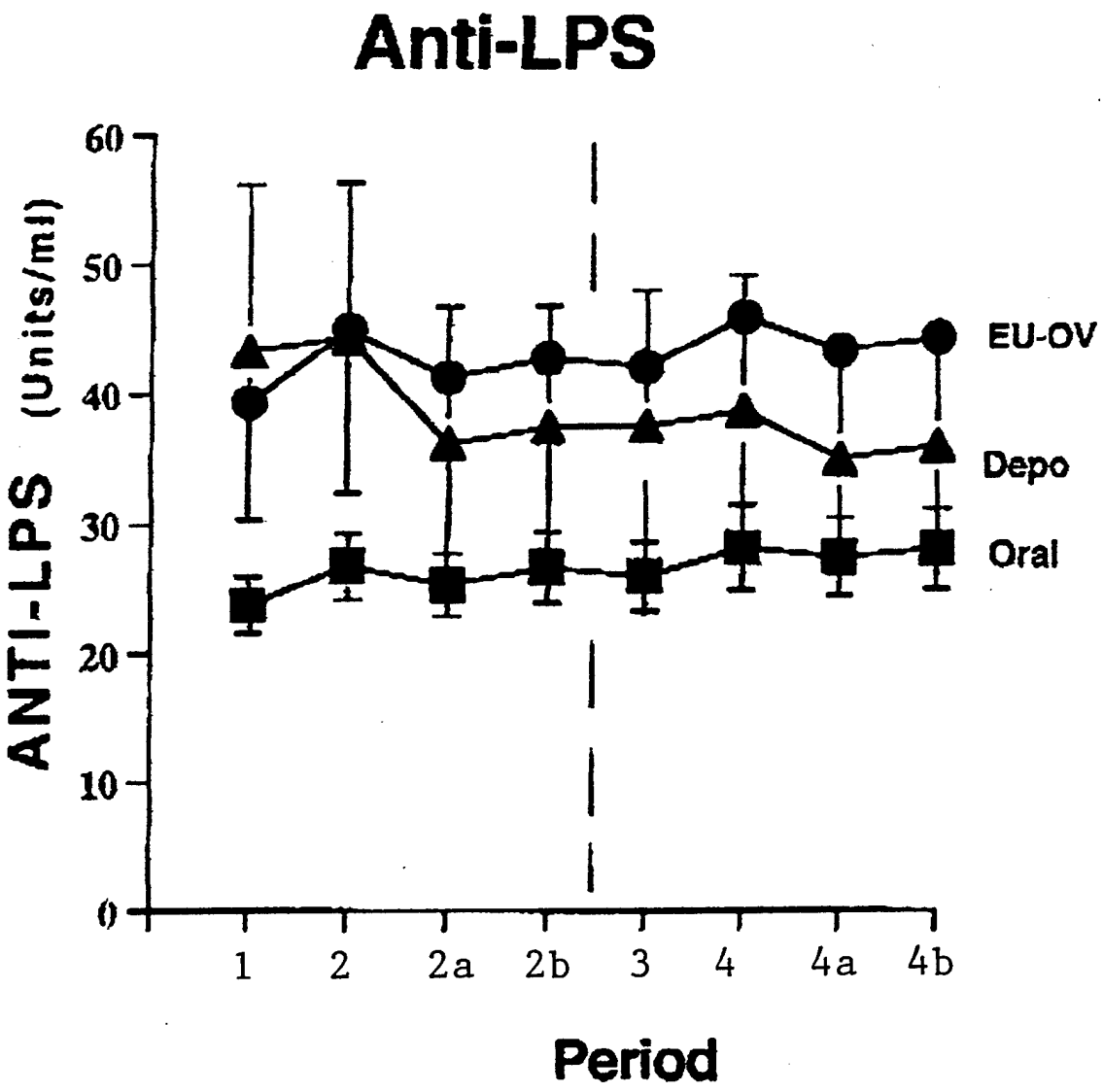
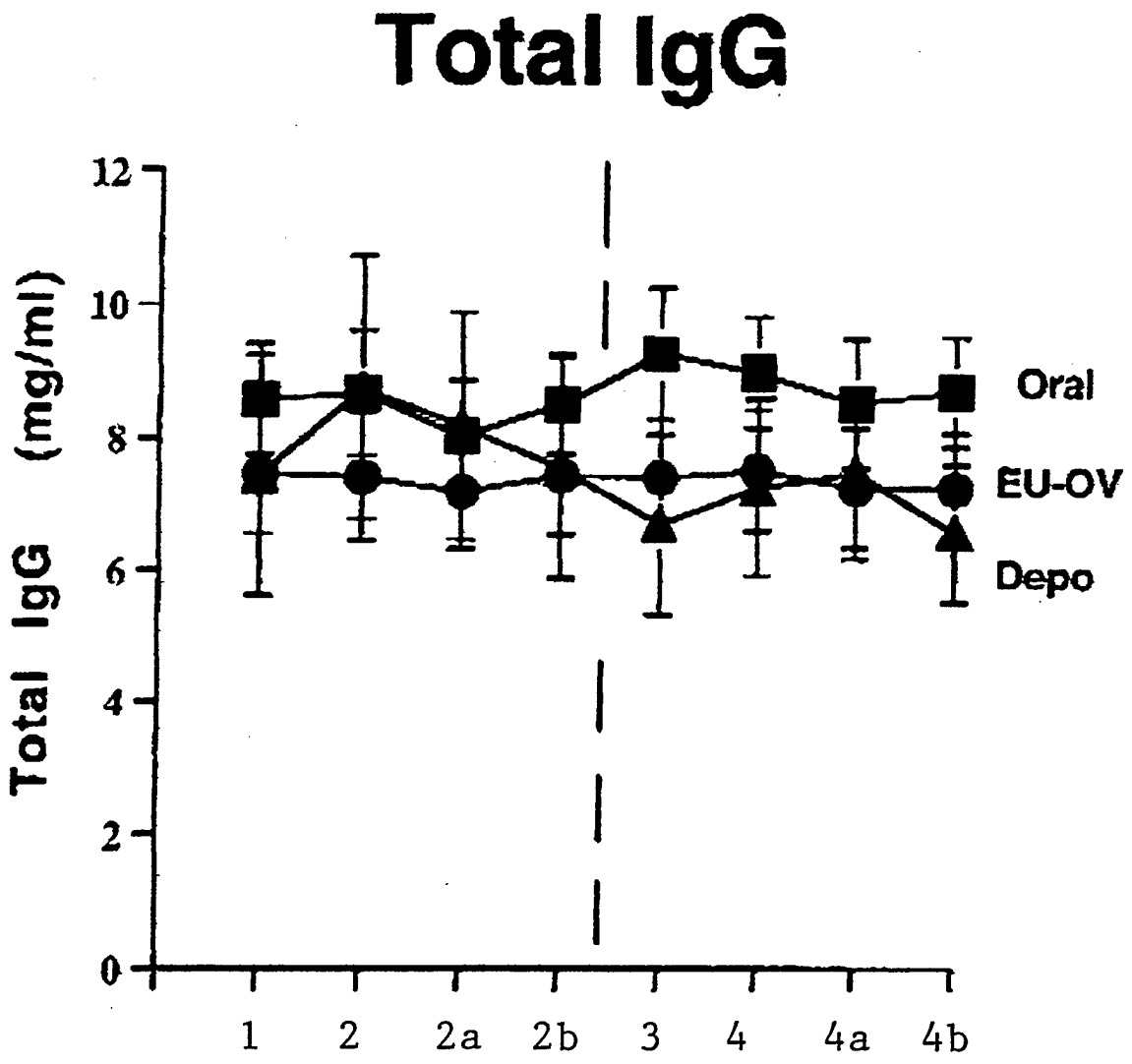

Anti-LPS
ANTI-LPS (Units/ml)
60
50
40
30
20
10
0
1
2
2a
2b
3
4
4a
4b
Period
EU-OV
Depo
Oral

Figure 6


Total IgG
Total IgG (mg/ml)
12
10
8
6
4
2
0
1
2
2a
2b
3
4
4a
4b
Oral
EU-OV
Depo

Key Research Accomplishments & Reportable Outcomes

1. Over 125 female volunteers were originally identified as potential test subjects, but hormonal and other physiological/clinical screening narrowed the pool to 36. These test subjects completed all phases of testing, in Years I through III.

2. Each of the physical training variables (Table 2) indicated that these three groups of test subjects were stronger, more physically fit, and had less body fat but more fat-free mass at the end of the 8-week physical training program.

3. Each of the variables measured during EHT tests (Table 3) indicated that these three groups of participants achieved heat acclimation during the 8-week training program. This conclusion is supported by EHT heart rate, rectal temperature, rating of perceived exertion, mean weighted skin temperature, and exercise time to exhaustion.

4. In terms of reproductive hormone trends, study participants responded to the training regimen unremarkably. The ovulatory status of the eumenorrheic group at post-training was similar to the pre-training period. Cycle length, follicular phase length, and luteal length appear to have remained unchanged. Hormonal responses of the contraceptive users also appeared to be unremarkable. The multiple stressors provided by eight weeks of physical training and heat exposure had no important effects on either menstrual or hormonal status.

5. In general, the circulating catecholamine results were unremarkable. Plasma epinephrine concentrations were unchanged among groups or across time. Plasma norepinephrine concentrations also were unchanged among groups, however the ORAL group showed a significant increase (pre-exercise to post-exercise) during the pre-acclimation testing, and pre-exercise values were significantly lower as a result of physical training. It is not clear why this lower post-training response was observed only in the ORAL group. Exercise training is known to contribute to lower plasma catecholamine concentrations.

6. Resting aldosterone concentrations were unremarkable, with similar concentrations among groups and across time. Thus, the combination of exercise training and heat acclimation did not impact circulating aldosterone in any of the three groups.

7. An original health concern of women soldiers during deployment was that the consumption of oral contraceptives might alter their immune systems, placing them at greater risk of infections. Preliminary findings (see Annual Report dated October, 1998) suggested that oral contraceptives activated humoral and cellular immune responses to exercise-heat stress. The entire data base of this study, however, now suggests that the effect of oral contraceptives on the immune system is small or physiologically unimportant.

Bibliography of Publications and Meeting Abstracts

At the time of this Final Report [October 21, 1999], no abstracts or manuscripts have been finalized or submitted to journals/conferences.

Personnel Who Received Funds From This Grant

Year I

Aresco, Dean
Armstrong, Lawrence
DeSouza, Mary Jane
Elliott, Tabatha
Herrara, Jorge
Johnson-Keith, Nicole
Maresh, Carl
Scheett, Timothy
Stoppani, James

Year II

Armstrong, Lawrence
DeSouza, Mary Jane
Elliott, Tabatha
Maresh, Carl
Scheett, Timothy
Stoppani, James

Year III

Armstrong, Lawrence
Bairos, Lynn
Blair, David
Bolster, Douglas
Collins, Valerie
DeSouza, Mary Jane
Elliott, Tabatha
Kane, Gregory
Maresh, Carl
VanHeest, Jaci

Personnel Who Received Graduate Degrees As a Result of this Grant

Ph.D. Degree

Nicole Johnson-Keith	- completed
Tabatha Elliott	- in progress

Master's Degree

Jennifer Holub	- completed
Jennifer Ormerod	- completed
Dean Aresco	- in progress
Timothy Bilodeau	- in progress
David Blair	- in progress

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